

**CLAIMS**

1. A gene construct comprising (i) a nucleotide sequence comprising the open reading frames corresponding to the polyprotein of the infectious bursal disease virus (IBDV) operatively bound to a nucleotide sequence comprising a first promoter and (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter, wherein said first promoter is different from said second promoter.

2. A gene construct according to claim 1, wherein said first promoter is a viral promoter and said second promoter is a viral promoter different from said first promoter.

3. A gene construct according to claim 1, comprising:

- (i) a nucleotide sequence comprising the open reading frames corresponding to the polyprotein IBDV operatively bound to a nucleotide sequence comprising a first promoter of a baculovirus, and
- (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter of a baculovirus,

wherein said first baculovirus promoter is different from said second baculovirus promoter.

4. A gene construct according to claim 3, wherein said first baculovirus promoter is selected from the group consisting of the promoter of the p10 protein of the baculovirus *Autographa californica* nucleopolyhedrovirus (AcMNV) and the promoter of the polyhedrin of the baculovirus AcMNPV.

5. A gene construct according to claim 3, wherein said second baculovirus promoter is selected from the group consisting of the promoter of the AcMNPV p10 protein and the promoter of the AcMNPV polyhedrin.

6. A gene construct according to claim 3, wherein said first baculovirus promoter is the promoter of the AcMNPV p10 protein and said second baculovirus promoter is the promoter of the AcMNPV polyhedrin; or wherein said first baculovirus promoter is the promoter of the AcMNPV polyhedrin and said second baculovirus promoter is the promoter of the AcMNPV p10 protein.

7. A gene construct according to claim 1, comprising the nucleotide sequence of SEQ ID NO: 1.

8. An expression system selected from:

- a) an expression system comprising a gene construct according to claim 1, operatively bound to transcription, and optionally translation, control elements; and
- b) an expression system comprising (1) a first gene construct, operatively bound to transcription, and optionally translation, control elements, wherein said first gene construct comprises a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first promoter, and (2) a second gene construct, operatively bound to transcription, and optionally translation, control elements, wherein said second gene construct comprises a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter.

9. An expression system according to claim 8, comprising a gene construct, operatively bound to transcription, and optionally translation, control elements, wherein said gene construct comprises (i) a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first baculovirus promoter and (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second baculovirus promoter, wherein said first baculovirus promoter is different from said second baculovirus promoter.

10. An expression system according to claim 8, comprising (1) a first gene construct, operatively bound to transcription, and optionally translation, control elements, said first gene construct comprising a nucleotide sequence comprising the open reading frames  
5 corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first baculovirus promoter, and (2) a second gene construct, operatively bound to transcription, and optionally translation, control elements, said second gene construct comprising a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second  
10 baculovirus promoter.

11. An expression system according to claim 10, wherein said first baculovirus promoter and said second baculovirus promoter are equal to or different from one another.

12. An expression system according to claim 8, selected from plasmids, bacmids, yeast artificial chromosomes (YACs), bacteria artificial chromosomes (BACs), P1 bacteriophage-based artificial chromosomes (PACs), cosmids and viruses, which can optionally contain a heterologous replication origin.

13. A host cell containing:

(A) a gene construct comprising (i) a nucleotide sequence comprising the open reading frames corresponding to the polyprotein of the infectious bursal disease virus (IBDV) operatively bound to a nucleotide sequence comprising a first promoter and (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1  
25 protein operatively bound to a nucleotide sequence comprising a second promoter, wherein said first promoter is different from said second promoter;

(B) the gene construct of (A) operatively bound to transcription, and optionally translation, control elements; or

(C) an expression system comprising (1) a first gene construct, operatively bound to  
30 transcription, and optionally translation, control elements, wherein said first gene construct comprises a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first promoter, and (2) a second gene construct, operatively bound to transcription, and optionally

translation, control elements, wherein said second gene construct comprises a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter.

5           14. A cell transformed, transfected or infected with an expression system according to claim 8.

          15. A cell according to claim 13, selected from the group consisting of animal cells and bacteria.

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          16. A cell according to claim 15, characterized in that it is the bacteria identified as DH5-pFBD/Poly-VP1 which is deposited in the CECT with deposit number CECT 5777.

          17. A cell according to claim 15, selected from the group consisting of insect cells, 15 bird cells and mammal cells.

          18. A dual recombinant baculovirus simultaneously expressing the IBDV polyprotein and the IBDV VP1 protein from (i) a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence 20 comprising a first baculovirus promoter, and from (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second baculovirus promoter, wherein said first baculovirus promoter is different from said second baculovirus promoter.

25           19. A process for the production of whole empty viral capsids of IBDV, comprising use of:

          (A) an expression system comprising a gene construct including (i) a nucleotide sequence comprising the open reading frames corresponding to the polyprotein of the infectious bursal disease virus (IBDV) operatively bound to a nucleotide sequence 30 comprising a first promoter and (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter, wherein said first promoter is different from said second

promoter, wherein said gene construct is operatively bound to transcription, and optionally translation, control elements;

(B) an expression system comprising (1) a first gene construct, operatively bound to transcription, and optionally translation, control elements, wherein said first gene construct  
5 comprises a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first promoter, and (2) a second gene construct, operatively bound to transcription, and optionally translation, control elements, wherein said second gene construct comprises a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein  
10 operatively bound to a nucleotide sequence comprising a second promoter; or

(C) a dual recombinant baculovirus simultaneously expressing the IBDV polyprotein and the IBDV VP1 protein from (i) a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first baculovirus promoter, and from (ii) a nucleotide sequence comprising the  
15 open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second baculovirus promoter, wherein said first baculovirus promoter is different from said second baculovirus promoter.

20. A process for the production of whole empty viral capsids of the infectious bursal  
20 disease virus (IBDV) [whole IBDV VLPs], comprising culturing a host cell as claimed in claim 13, and if desired, recovering said whole IBDV VLPs.

21. A process according to claim 20, wherein said host cell is a cell transformed, transfected or infected with an expression system comprising a gene construct comprising (i)  
25 a nucleotide sequence comprising the open reading frames corresponding to said IBDV polyprotein operatively bound to a nucleotide sequence comprising a first promoter and (ii) a nucleotide sequence comprising the open reading frame corresponding to said IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter, wherein said first promoter is different from said first promoter.

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22. A process according to claim 20, wherein said host cell is a cell transformed, transfected or infected with an expression system comprising a gene construct comprising (1) a first gene construct comprising a nucleotide sequence comprising the open reading frames

corresponding to said IBDV polyprotein and (2) a second gene construct comprising a nucleotide sequence comprising the open reading frame corresponding to said IBDV VP1 protein, each one of said nucleotide sequences comprising the ORFS corresponding to the viral polyprotein and to the IBDV VP1 protein being under the control of respective  
5 nucleotide sequences comprising respective promoters, equal to or different from one another.

23. A process according to claim 21, wherein said host cell is an insect cell.

10 24. A process according to claim 20, wherein said host cell is an insect cell, comprising the steps of:

a) preparing an expression system made up of a dual recombinant baculovirus comprising a gene construct comprising (i) a nucleotide sequence comprising the  
15 open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first baculovirus promoter, said gene construct being operatively bound to transcription, and optionally translation, control elements, and (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide  
20 sequence comprising a second baculovirus promoter, said gene construct being operatively bound to transcription, and optionally translation, control elements, wherein said baculovirus promoter is different from said second baculovirus promoter;

25 b) infecting insect cells with said expression system prepared in step a);

c) culturing the infected insect cells obtained in step b) under conditions allowing the expression of the recombinant proteins and their assembly to form whole IBDV VLPs; and

30 d) if desired, isolating and optionally purifying said whole IBDV VLPs.

25. A process according to claim 20, wherein said host cell is an insect cell, comprising the steps of:

- 5 a) preparing an expression system made up of (1) a first recombinant baculovirus comprising a gene construct comprising a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a baculovirus promoter, said gene construct being operatively bound to transcription, and optionally translation, control elements, and of (2) a second recombinant baculovirus comprising a gene construct comprising a nucleotide  
10 sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a promoter of a baculovirus, said gene construct being operatively bound to transcription, and optionally translation, control elements;
- 15 b) infecting insect cells with said expression system prepared in step a);
- c) culturing the infected insect cells obtained in step b) under conditions allowing the expression of the recombinant proteins and their assembly to form whole IBDV VLPs; and
- 20 d) if desired, isolating and optionally purifying said whole IBDV VLPs.

26. Whole empty capsids of the infectious bursal disease virus (IBDV) [whole IBDV VLPs], obtained according to the process of claim 20.

25 27. Whole empty capsids of the infectious bursal disease virus (IBDV) [whole IBDV VLPs], characterized by containing the VPX, VP2, VP3 and VP1 proteins of IBDV.

30 28. A therapeutic composition comprising whole empty capsids of the infectious bursal disease virus (IBDV) [whole IBDV VLPs] containing the VPX, VP2, VP3 and VP1 proteins of IBDV.

29. A method of combating avian infectious bursal disease, comprising administering

to an avian subject a vaccine comprising whole empty capsids of the infectious bursal disease virus (IBDV) [whole IBDV VLPs] containing the VPX, VP2, VP3 and VP1 proteins of IBDV.

5           30. A gene therapy vector including whole empty capsid of the infectious bursal disease virus (IBDV) [whole IBDV VLP] containing the VPX, VP2, VP3 and VP1 proteins of IBDV.

10           31. A vaccine comprising a therapeutically effective amount of whole empty capsids of IBDV [whole IBDV VLPs], containing the VPX, VP2, VP3 and VP1 proteins of IBDV, optionally combined with one or more pharmaceutically acceptable adjuvants and/or vehicles.

15           32. A vaccine according to claim 31, for protecting birds from the infection caused by IBDV.

            33. A vaccine according to claim 32, wherein said birds are selected from the group formed by chickens, turkeys, geese, ganders, pheasants, partridges and ostriches.

20           34. A vaccine for protecting chickens from an infection caused by infectious bursal disease virus (IBDV), comprising a therapeutically effective amount of whole empty capsids of IBDV, whole IBDV VLPs, containing the VPX, VP2, VP3 and VP1 proteins of IBDV, optionally combined with one or more pharmaceutically acceptable adjuvants and/or vehicles.

25           35. A process for obtaining a dual recombinant baculovirus allowing the simultaneous expression in insect cells of the polyprotein of the infectious bursal disease virus (IBDV) and of the IBDV VP1 protein from two independent open reading frames and each one of them controlled by a different baculovirus promoter, comprising:

30           a) constructing a plasmid carrying a gene construct containing (i) a nucleotide sequence comprising the open reading frames corresponding to the IBDV polyprotein operatively bound to a nucleotide sequence comprising a first promoter of a



baculovirus, and (ii) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP1 protein operatively bound to a nucleotide sequence comprising a second promoter of a baculovirus, wherein said first baculovirus promoter is different from said second baculovirus promoter;

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b) obtaining a recombinant bacmid, allowing the simultaneous expression during its replicative cycle of the polyprotein and the IBDV VP1 protein under transcriptional control of said baculovirus promoters, by means of the transformation of competent bacteria with the plasmid obtained in a); and

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c) obtaining a dual recombinant baculovirus, allowing the simultaneous expression of the open reading frames corresponding to the polyprotein and the IBDV VP1 protein under transcriptional control of said baculovirus promoters, by means of transformation of insect cells with the recombinant bacmid of b).

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36. A process according to claim 35, wherein:

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- said first baculovirus promoter is the promoter of the AcMNV p10 protein and said second baculovirus promoter is the promoter of the AcMNPV polyhedrin, or vice versa;

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- the plasmid obtained in a) is the one identified as pFBD/Poly-VP1;
- the competent bacteria of b) are *E. coli* DH10Bac;
- the recombinant bacmid obtained in b) is the one identified as Bac/pFBD/Poly-VP1; and
- the recombinant baculovirus obtained is the one identified as FBD/Poly-VP1.

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37. A process according to claim 35, further comprising the infection of insect cells with the dual recombinant baculovirus obtained in step c).

38. A process according to claim 37, wherein said insect cells are H5 or *Spodoptera frugiperda* Sf9 cells.